FEBS Letters 580 (2006) 2661–2666

Chitooligosaccharides inhibit activation and expression of matrix metalloproteinase-2 in human dermal fibroblasts

Moon-Moo Kim[a], Se-Kwon Kim[a,b,*]

[a] *Marine Bioprocess Research Center, Pukyong National University, Busan 608-737, Republic of Korea*
[b] *Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea*

Received 3 March 2006; revised 18 March 2006; accepted 7 April 2006

Available online 21 April 2006

Edited by Gianni Cesareni

Abstract **Recently, much attention has been given to chitosan and its hydrolyzed products due to their diverse biological activities. For the first time here we report the inhibitory effect of chitooligosaccharides (COS) on activation and expression of matrix metalloproteinase-2 (MMP-2) in primary human dermal fibroblasts (HDFs). COS with 3–5 kDa exhibited the highest inhibitory effect on MMP-2 activity in HDFs assessed by gelatin zymography. Interestingly, protein expression of MMP-2 was also inhibited by COS with same molecular weight. This inhibition was caused by the decrease of the gene expression and transcriptional activity of MMP-2. Furthermore, it was found that COS repressed the gene expression of c-fos, a part of AP-1 transcription factor. These results suggest that COS may play an important role in the prevention and treatment of MMP-2 mediated several health problems such as metastasis and wrinkle formation.**

Keywords: Matrix metalloproteinase-2; Chitooligosaccharide; Gelatin zymography; Human dermal fibroblasts; Gene reporter assay

1. Introduction

Matrix metalloproteinases (MMPs) are a family of secreted or transmembrane zinc-endopeptidases [1] that are capable of digesting extracellular matrix (ECM), such as fibrillar and non-fibrillar collagens, fibronectin, laminin, elastin and basement membrane glycoproteins under physiological conditions [2]. MMPs play an important role not only in physiologic degradation of ECM mediating tissue morohogenesis, tissue repair, and angiogenesis but also in pathologic conditions characterized by excessive degradation of ECM such as chronic inflammation, wrinkle formation, arthritis, osteoporosis, periodontal disease, tumor invasion and metastasis. Direct evidence for the involvement of distinct MMPs in tumor growth and invasion has been revealed by studies with either MMP-2 knockout mice having reduced melanoma tumor progression and angiogenesis [3]. Therefore, inhibition of MMP activities in the extracellular space has been extensively studied as an approach to inhibit metastasis and wrinkle formation. At present, several MMP inhibitors are under clinical trials and most of these MMP inhibitors are synthetic peptides, chemically modified tetracyclines, bisphosphonates or compounds isolated from natural sources. However, most of these drugs are reported to exert side effects such as, musculoskeletal pain in tendons and joints [4]. Therefore, we have made an effort to screen safe and effective MMP inhibitors and found for the first time that chitooligosaccharides (COS) can inhibit MMP-2 activation and expression. Chitosan, a non-toxic biopolymer, is a partially deacetylated polymer of *N*-acetyl glucosamine, which is obtained after alkaline deacetylation of the chitin derived from the exoskeletons of crustaceans and arthropods. It has received considerable attention for its commercial applications in biomedical and chemical fields because of its biological functions such as antitumor activity [5], antimicrobial activity [6], antimutagenic activity [7] and immuno-enhancing effects [8]. COS, partially hydrolyzed products of chitosan are of great interest in pharmaceutical and medicinal applications due to high solubility and nontoxicity. However, little information on the inhibitory effect of COS on MMP expression is available until now. Therefore, in the present work we carried out a detailed study to investigate the inhibitory effects of COS on MMP-2 expression in normal human cells.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation, 93%; average molecular weight, 280 kDa; viscosity, 13 cP) prepared from crab shell chitin was donated by Kitto Life Co. (Seoul, Korea). Chitosanase from *Bacillus* sp. (35 000 U/g of protein) was purchased from Amicogen Co. (Jinju, Korea). Ultrafiltration membrane (UF) reactor system (Minitan) and membranes, for the fractionations of COS were purchased from Millipore Co. (Bedford, MA, USA). Dulbecco's modified Eagle's medium (DMEM), trypsin–EDTA, penicillin/streptomycin/amphotericin (10 000 U/ml, 10 000 μg/ml, and 2500 μg/ml, respectively) and fetal bovine serum (FBS) were obtained from Gibco BRL, Life Technologies (NY, USA). Human dermal fibroblasts were kindly donated by LG HG&CM Research Institute (Daejeon, Korea). MTT reagent, gelatin, agarose, PMA (phorbol 12-myristate 13-acetate) and other materials were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of COS using UF membrane bioreactor

Chitosan solution (1%) was prepared by dispersing 100 g of chitosan in 1.0 L of distilled water while stirring with 550 ml of 1.0 M lactic acid and making the final volume up to 10 L with distilled water. The pH of

*Corresponding author. Fax: +82 51 628 8147.
E-mail addresses: mmkim@kaist.ac.kr (M.-M. Kim), sknkim@pknu. ac.kr (S.-K. Kim).

Abbreviations: MMP-2, matrix metalloproteinase-2; COS, chitooligosaccharides; ECM, extracellular matrix; HDFs, human dermal fibroblasts; PMA, phorbol 12-myristate 13-acetate

0014-5793/$32.00
doi:10.1016/j.febslet.2006.04.015

the solution was adjusted to 5.5 using saturated $NaHCO_3$ solution. COS were prepared by continuous hydrolysis of chitosan according to our previous method, in an UF membrane reactor system connected to an immobilized enzyme column reactor in which chitosanase (from *Bacillus* sp., Amicogen Co.) was immobilized [9]. Four UF membranes with different molecular weight cut-offs (MWCO of 10, 5, 3, and 1 kDa) were used to separate COS into five different molecular weight fractionations. Fractionated COS were separately dialyzed using 10, 5, 3, and 1 kDa molecular weight cut-off dialysis membranes (Pierce Biotechnology Inc, Rockfod, IL, USA) and lyophilized.

2.3. Cell culture

Cell lines were separately grown as monolayers at 5% CO_2 and 37 °C humidified atmosphere using appropriate media supplemented with 10% fetal bovine serum, 2 mM glutamine and 100 μg/ml penicillin–streptomycin. DMEM was used as the culture medium for human dermal fibroblasts (HDFs) cultured primarily from human fetal skin. Cells were passaged 3 times a week by treating with trypsin–EDTA and used for experiments after 5 passages.

2.4. MTT assay

Cytotoxic levels of COS on HDFs were measured using MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) method as described by Hansen et al. [10].

2.5. Gelatin zymography

Expression and activation of MMP-2 were determined by zymography as described previously [11] in the presence or absence of COS. Conditioned medium containing same amount of total proteins was loaded into each well of polyacrylamide gels containing 1.5 mg/ml gelatin and electrophoresed under non-reducing conditions. Depending on the purpose, different protein amounts for each experiment were used for gelatin zymography analysis. Gelatinolytic bands were observed as clear zones against the blue background and the intensity of the bands was estimated using ImageMaster Software (Amersham Pharmacia Bioscience, NJ, USA).

2.6. Western blot analysis

Western blotting was performed according to standard procedures. Briefly, HDFs were lysed in lysis buffer containing 50 mM Tris–HCl, pH 7.5, 0.4% Nonidet P-40, 120 mM NaCl, 1.5 mM $MgCl_2$, 2 mM phenylmethylsulfonyl fluoride, 80 μg/ml leupeptin, 3 mM NaF and 1 mM DTT at 4 °C for 30 min. Cell lysates (10 μg) were resolved on a 4–20% Novex® gradient gel (Invitrogen, USA), electrotransferred onto a nitrocellulose membrane, and blocked with 10% skim milk. Proform of anti-MMP-2 primary monoclonal antibodies (Cat. No. MAB13405, Chemicon, CA, USA) were used to detect pro-MMP-2 protein using chemiluminescent ECL assay kit (Amersham Pharmacia Biosciences, NJ, USA) according to the manufacturer's instructions. Western blot bands were quantified using ImageMaster software (Amersham Pharmacia Biosciences, NJ, USA).

2.7. Transfection and reporter gene assay

Human dermal fibroblasts were seeded in 6-well plates and incubated at 37 °C. At about 70–80% of confluency, cells were washed with DMEM and incubated with DMEM without serum and antibiotics for 5 h. Then the cells were transfected with DNA mixture containing 1 μg of MMP-2 promoter containing pGL3 reporter vector and 300 ng of pcDNA 3.1 which adjusts a total amount of DNA per well by Lipofectamine™ reagent (Invitrogen, USA) as instructed by the manufacturer. After 72 h of incubation, cells were lysed and luciferase activity was measured using a luminometer (Tecan Austria GmbH, Austria). The luciferase activity was normalized to transfection efficiency, monitored by β-galactosidase activity using *o*-nitrophenyl *β*-galactopyranoside as a substrate. Data were expressed as means ± S.E. of the mean ($n = 3$). Values were analyzed statistically relative to the control using Student's *t*-test.

2.8. RT-PCR

For RT-PCR, 1 μg of total RNA prepared from HDFs was reverse-transcribed to generate first strand cDNA using AMV reverse transcriptase, (USB coporation, OH, USA). Synthesized cDNA was used as a template in PCR using *Taq* polymerase, (USB corporation, OH,

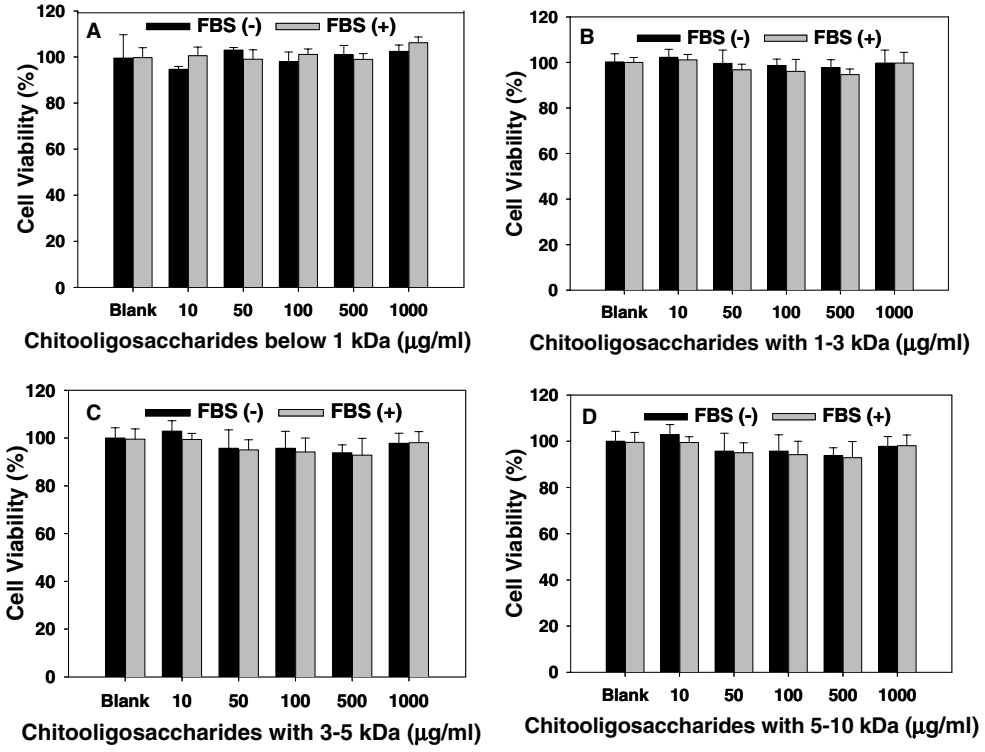

Fig. 1. Effect of COS with different molecular weights on viability of HDFs in the presence or absence of FBS. Cells were treated with COS with different molecular weights (A, <1 kDa; B, 1–3 kDa; C, 3–5 kDa; D, 5–10 kDa) and cell viability was determined by MTT assay after 24 h. Data are given as means of values ± S.D. from three independent experiments.

USA) with pairs of primers for MMP-2 (forward primer: 5′-GTGCTGAAGGACACACTAAAGAAGA-3′, reverse primer: 5′-TTGCCATCCTTCTCAAAGTTGTAGG-3′), c-fos (forward primer: 5′-CTACGAGGCGTCATCCTCCCG-3′, reverse primer: 5′-AGCTCCCTCCTCCGGTTGCGGCAT-3′) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (forward primer: 5′-TGAAG GTCGGTGTGAACGGATTTGGC-3′, reverse primer: 5′-CAT-GTAGGCCATGAGGTCCACCAC-3′). PCR products were then separated on a 2% agarose gel and visualized by ethidium bromide staining.

2.9. Statistical analysis

Comparisons of all data were performed using two-tailed, unpaired Student's *t*-test. A P value less than 0.05 was considered statistically significant. Data are expressed as means ± S.E.

3. Results

3.1. Effect of COS with different molecular weights on proliferation of HDFs

The purpose of present study was to investigate the inhibitory effects of COS with different molecular weights on MMP-2 expression and activation. Therefore, in the first place, non-cytotoxic concentrations of COS on normal HDFs in the presence or absence of FBS were determined by performing MTT assay for MMP inhibition studies. Interestingly, as shown in Fig. 1, all COS used in this study did not exert any cytotoxic effect on HDFs even at the highest tested concentration (1000 μg/ml) in the presence or absence of FBS.

3.2. Concentration and time dependent inhibitory effects of COS with different molecular weights on MMP-2

Based on the above results, further studies were carried out to examine the inhibitory effect of COS with different molecular weights on MMP-2 activation and expression in HDFs. Conditioned medium of HDFs treated with COS with different molecular weights was used for gelatin zymography. It was observed clearly that latent form of MMP-2 (proMMP-2) was converted into its active form by PMA treatment (Fig. 2). All COS above 50 μg/ml exhibited a marked inhibitory effect on MMP-2 activation in HDFs compared with PMA treatment groups. To clarify the inhibitory effect of COS on MMP-2, time dependent inhibitory effect was investigated. As shown in Fig. 3, treatment with PMA enhanced proMMP-2 activity significantly compared with blank group ($P < 0.01$). Moreover, proMMP-2 activity was clearly increased with incubation time in both PMA and COS treatment groups below 1 kDa. However, inhibitory effect of COS above

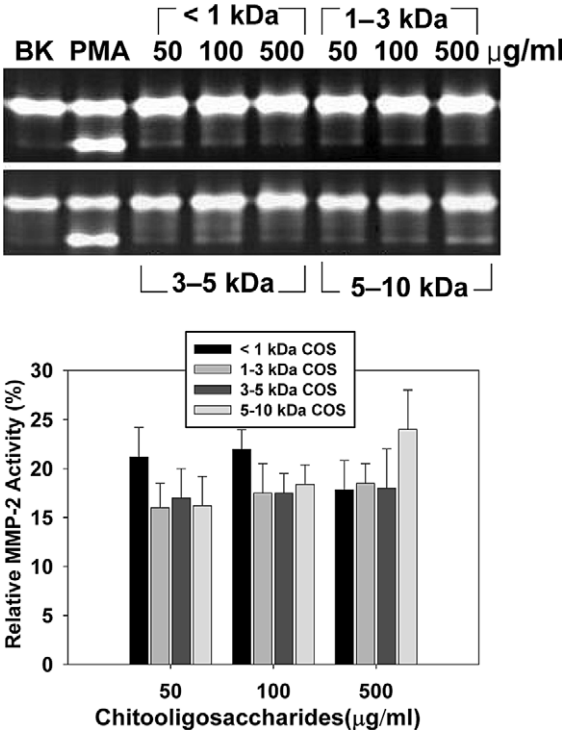

Fig. 2. Effect of COS with different molecular weights on activity of MMP-2 in HDFs with treatment concentration. HDFs stimulated with 100 ng/ml of PMA to induce MMP expression were treated with COS with different molecular weights at 50, 100 and 500 μg/ml under serum-free conditions for 7 days. Upper panel illustrates MMP-2 activity in conditioned media as determined by gelatin zymography. Lower panel represents respective relative MMP-2 activity of each treatment calculated considering 100% activity in PMA treated group. Data are given as means of values ± S.D. from three independent experiments.

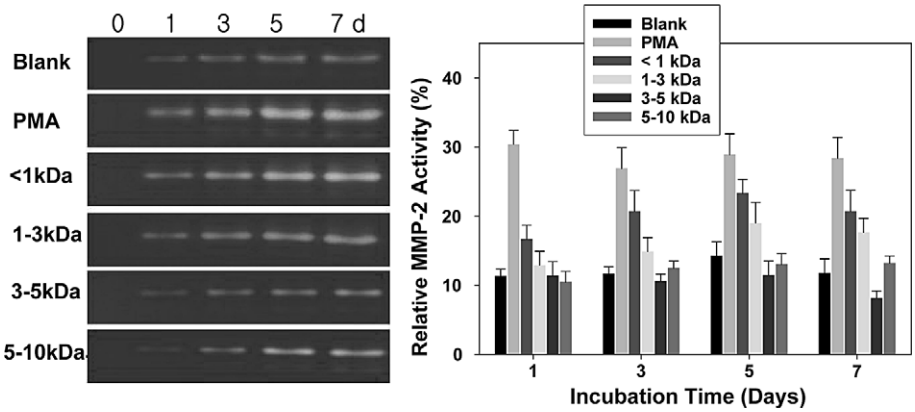

Fig. 3. Effect of COS with different molecular weights on activity of MMP-2 in HDFs with incubation time. HDFs stimulated with 100 ng/ml of PMA to induce MMP expression were treated with COS with different molecular weights under serum-free conditions for 1, 3, 5 and 7 days. MMP-2 activity in conditioned media was determined by gelatin zymography as described in the text. Values are expressed as relative MMP-2 activities using the following equation. Relative MMP-2 activity (%) = (intensity of a band/total intensity of bands obtained for a day) × 100. Each day was considered as an independent group. Data are given as means of values ± S.D. from three independent experiments.

1 kDa on proMMP-2 activity was significantly increased with incubation time ($P < 0.01$). Furthermore, among all molecular weight fractions of COS, 3–5 kDa fraction exerted the highest inhibitory effect on proMMP-2 activity.

3.3. Inhibitory effects of COS with different molecular weights on proMMP-2 protein expression in HDFs

To determine the inhibitory effect of COS with different molecular weights on the protein expression level of proMMP-2, Western blot analysis was performed in HDFs. As shown in Fig. 4, PMA treatment significantly increased the expression level of proMMP-2 compared with blank group. The expression level of proMMP-2 protein was decreased with the treatment of COS with all molecular weights, compared with PMA treatment group. However, the highest inhibition was observed with 3–5 kDa of COS, and that inhibition was significantly different ($P < 0.01$) from those of the other molecular weights.

3.4. Transcriptional activation of MMP-2 in HDFs is inhibited by COS

Furthermore, it was examined whether transcriptional activity of MMP-2 is affected by COS with different molecular weights using a reporter construct including MMP-2-luciferase gene. As shown in Fig. 5, transcriptional activity of MMP-2 gene was increased significantly ($P < 0.01$) in control group treated with PMA, compared to the untreated blank group. However, the transcriptional activity of MMP-2 gene induced by PMA was considerably repressed by treatment with COS. Especially, COS with 3–5 kDa exhibited the highest inhibitory effect on transcriptional activity of MMP-2 gene. The inhibitory effect on transcriptional activity of MMP-2 gene was increased with increasing concentrations of COS. In addition, all COS at 100 μg/ml significantly ($P < 0.05$) repressed transcriptional activity of MMP-2 gene.

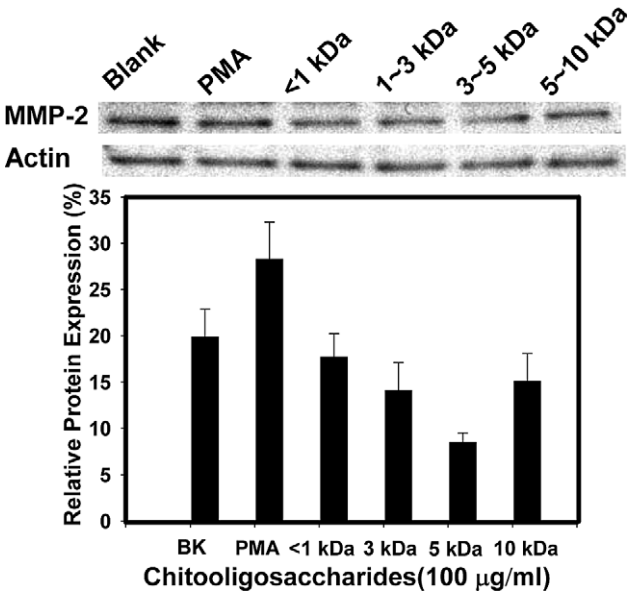

Fig. 4. Western blot analysis of protein expression of proMMP-2 in HDFs after treatment with different molecular weights of COS. Western blot analysis of cell lysates was performed using proform of MMP-2 monoclonal antibodies as indicated. Lower panel represents respective relative protein expression as percentage. Values are expressed as relative protein expression using the following equation. Relative protein expression (%) = (intensity of a band/total intensity of bands) × 100. Expression of β-actin protein was used as the control for normalization of proMMP-2 protein.

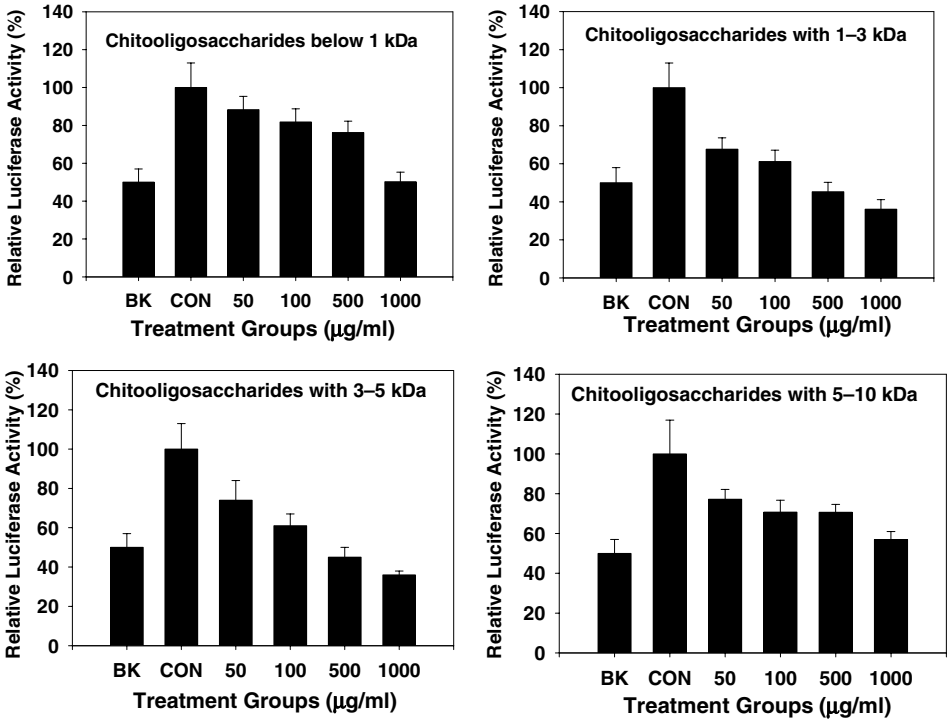

Fig. 5. Effects of COS with different molecular weights on the transcriptional activity of MMP-2 promoter activation in HDFs. Transcriptional activity of MMP-2 was measured by luciferase assay in HDFs after cotransfection with pMMP-2 reporter vector and pcDNA 3.1. Data represent the mean ± S.E. of at least three independent experiments. Level of significance was identified statistically (* indicates $P < 0.01$) using Student's t test.

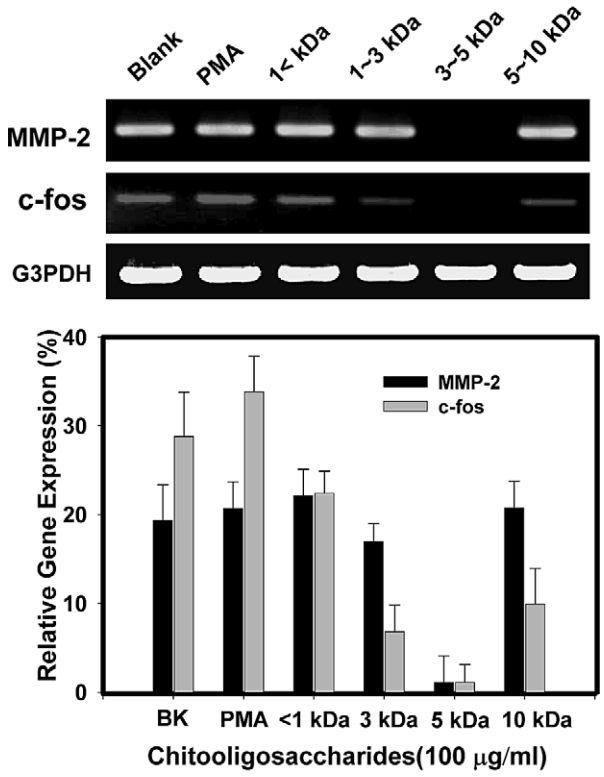

Fig. 6. Effects of COS with different molecular weights on gene expression of MMP-2 and c-fos in HDFs. Cells were treated with 100 μg/ml of COS having different molecular weights and gene expression levels were determined by RT-PCR. Upper panel illustrates PCR products of MMP-2, c-fos and G3PDH genes after electrophoresis on 2% agarose gel. Lower panel represents densiometrically determined respective relative gene expression levels normalized to G3PDH. Values were expressed as relative gene expression using the following equation. Relative gene expression (%) = (intensity of a band/total intensity of bands) × 100. Data represent the means ± S.E. of at least three independent experiments. Level of significance was identified statistically (* indicates $P < 0.01$) using Student's t test.

3.5. Effects of COS with different molecular weights on gene expression of MMP-2 and c-fos in HDFs

To confirm the inhibitory effect on gene expression of MMP-2, we performed RT-PCR analysis using total cellular RNA prepared from HDFs treated with different molecular weights of COS. As shown in Fig. 6, the gene expression of MMP-2 was completely inhibited by COS with 3–5 kDa. There was no significant difference in MMP-2 gene expression among other groups. Further, MMP-2 gene expression is known to be regulated via AP-1 transcription factor that binds to promoter of all MMPs. Therefore, we investigated whether COSs exert any inhibitory effect on AP-1. For that, we tested the gene expression of c-fos, a part of AP-1 transcription factor in the presence of COS. All COS above 1 kDa exhibited inhibitory effects on c-fos gene expression. Moreover, the highest inhibitory effect was observed with 3–5 kDa of COS.

4. Discussion

Recently, much attention has been given to the use of chitosan as a wound healing agent, antimicrobial agent, skin grafting template and drug delivery vehicle. However, these biological activities remain unclear in human. In this study, the inhibitory effect of COS with different molecular weights on the expression and activation of MMP-2 was evaluated in HDFs. COS with different molecular weights did not exert any cytotoxic effect on normal human cells, and this was supported by the fact that COS have long been utilized as functional foods in Korea. The broad specificity of MMP-2 may play a role in the regulation of various cellular activities. MMP-2 (gelatinase A, 72 kDa type IV collagenase) is a secreted endopeptidase able to hydrolyze several components of the extracellular matrix including basement membrane collagen IV and has been associated with tumor invasion and metastasis [12,13].

To examine whether COS inhibit MMP-2 expression and activation in HDFs, gelatin zymography was carried out. We observed that PMA treatment could stimulate MMP-2 expression and activation in HDFs. Moreover, this observation was consistent with previous report that MMP-2 is secreted in a latent form that is activated by a sequential cleavage of the N-terminal propeptide domain [14]. In this study, for the first time we found that COS with different molecular weights could inhibit activation of proMMP-2. Chitosan has been known to have an effective binding capacity for Zn^{2+} and used in commercial chelating ion-exchange resins [15]. Therefore, it is possible that this inhibitory effect is due to the chelating ability of COS on Zn^{2+} that is vital for the activity of MMP-2. We further investigated whether COS can inhibit MMP-2 protein expression in HDFs. Western blot analysis using proMMP-2 monoclonal antibody clearly confirmed that COS can inhibit protein expression of proMMP-2 in HDFs. Furthermore, COS with 3–5 kDa exhibited the highest inhibitory effect on protein expression of proMMP-2 and that was consistent with the results of gelatin zymography analysis over time. Previous research has shown that carbohydrates such as fucose-rich polysaccharide can be an efficient inhibitor capable of down-regulating the expression and activity of MMP-2 with potential therapeutic applications in age-related pathologies accompanied by tissue loss [16]. Based on the results of our experiment, we confirmed that MMP-2 expression and activation could be inhibited by COS in HDFs. Transcriptional regulation has an important role in the synthesis of MMP-2. Therefore, to investigate whether COS could affect transcriptional activity of MMP-2 in HDFs, reporter gene assay using pMMP-2-luciferase construct was carried out. It was observed that COS with 3–5 kDa could repress the transcriptional activity of MMP-2 in HDFs, which was consistent with the results of RT-PCR analysis. This observation can be further confirmed by the finding that gene expression level of c-fos was decreased by COS with 3–5 kDa. The promoter of MMP-2 gene is notable for the absence of the conserved proximal AP-1 site, and MMP-2 in contrast with most members of the MMP gene family is not regulated by AP-1 [17]. However, previous report has shown that there was a correlation between c-fos and MMP-2 gene expression [18] and presence of AP-1 binding site in promoter of MMP-2 [19]. It has also reported that c-fos gene may play an important role in the regulation of MMP-2 gene expression through activation of MT1-MMP [20]. In conclusion, our findings provide the first experimen-

tal evidence that COS inhibit activation and expression of MMP-2 in HDFs. These results suggest that COS may play an important role in the prevention and treatment of MMP-2 mediated several health problems such as metastasis and wrinkle formation.

Acknowledgments: The authors acknowledge Marine Bioprocess Research Center of Marine Bio 21 Project, funded by the Ministry of Maritime Affairs and Fisheries, Republic of Korea, for the support provided through the research grant, B-2005-02. We thank Dr. Sang-Oh Yoon (KAIST, Taejeon, Korea) for MMP-2 reporter vector.

References

[1] Kohn, E.C., Jacobs, W., Kim, Y.S., Alessandro, R., Stetler-Stevenson, W.G. and Liotta, L.A. (1994) Calcium influx modulates expression of matrix metalloproteinase-2 (72-kDa type IV collagenase, gelatinase A). J. Biol. Chem. 269, 21505–21511.

[2] Chakrabarti, S. and Patel, K.D. (2005) Matrix metalloproteinase-2 (MMP-2) and MMP-9 in pulmonary pathology. Exp. Lung Res. 31, 599–621.

[3] Itoh, T., Tanioka, M., Yoshida, H., Yoshioka, T., Nishimoto, H. and Itohara, S. (1998) Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res. 58, 1048–1051.

[4] Nelson, A.R., Fingleton, B., Rotherberg, M.L. and Matrisian, L.M. (2000) Matrix metalloproteinases: biologic activity and clinical implications. J. Clin. Oncol. 18, 1135–1149.

[5] Jeon, Y.J. and Kim, S.K. (2002) Antitumor activity of chitosan oligosaccharides produced in ultrafiltration membrane reactor system. J. Microbiol. Biotechnol. 12, 503–507.

[6] Kim, S.K., Jeon, Y.J. and Zan, H.C. (2000) Antibacterial effect of chitooligosaccharides with different molecular weights prepared using membrane bioreactor. J. Chitin Chitosan 5, 1–8.

[7] Shon, Y.H., Ha, Y.M., Jeong, T.R., Kim, C.H. and Nam, K.S. (2001) Modifying action of chitosan oligosaccharide on 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)-induced mutagenesis. J. Biochem. Mol. Biol. 34, 90–94.

[8] Jeon, Y.J. and Kim, S.K. (2001) Potential immuno-stimulating effect of antitumoral fraction of chitosan oligosaccharides. J. Chitin Chitosan 6, 163–167.

[9] Jeon, Y.J. and Kim, S.K. (2000) Continuous production of chitooligosaccharides using a dual reactor system. Process Biochem. 35, 623–632.

[10] Hansen, M.B., Nielsen, S.E. and Berg, K. (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Meth. 119, 203–210.

[11] Hrabec, E., Strek, M., Nowak, D., Greger, J., Suwalski, M. and Hrabec, Z. (2002) Activity of type IV collagenases (MMP-2 and MMP-9) in primary pulmonary carcinomas: a quantitative analysis. J. Cancer Res. Clin. 128, 197–204.

[12] Liotta, L.A., Steeg, P.A. and Stetler-Stevenson, W.G. (1991) Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 64, 327–336.

[13] Aimes, R.T. and Quigley, J.P. (1995) Matrix metalloproteinase-2 is an interstitial collagenase. J. Biol. Chem. 270, 5872–5876.

[14] Strongin, A.Y., Marmer, B.L., Grant, G.A. and Goldberg, G.I. (1993) A matrix metalloproteinase expressed on the surface of invasive tumour cells. J. Biol. Chem. 268, 14033–14039.

[15] Masri, M.S., Reuter, F.W. and Fiedman, M. (1974) Binding of metal cations by natural substances. J. Appl. Polym. Sci. 18, 675–681.

[16] Isnard, N., Peterszegi, G., Robert, A.M. and Robert, L. (2002) Regulation of elastase-type endopeptidase activity, MMP-2 and MMP-9 expression and activation in human dermal fibroblasts by fucose and a fucose-rich polysaccharide. Biomed. Pharmacother. 56, 258–264.

[17] Vincenti, M.P. (2001) The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. Transcriptional and posttranscriptional regulation, signal transduction and cell-type-specifc expression. Methods Mol. Biol. 151, 121–148.

[18] Naruo, S., Kanayama, H., Aki, M. and Kagawa, S. (1993) Gene expressions of type IV collagenase and tissue inhibitor of metalloproteinases (TIMP) in human bladder cancers. Nippon Hinyokika Gakkai Zasshi 84, 841–850.

[19] Bergman, M.R., Cheng, S., Honbo, N., Piacentini, L., Karliner, J.S. and Lovett, D.H. (2003) A functional activating protein 1 (AP-1) site regulates matrix metalloproteinase 2 (MMP-2) transcription by cardiac cells through interactions with JunB-Fra1 and JunB-FosB heterodimers. Biochem. J. 369, 485–496.

[20] Okada, A., Tomasetto, C., Lutz, Y., Bellocq, J.P., Rio, M.C. and Basset, P. (1997) Expression of matrix metalloproteinases during rat skin wound healing: evidence that membrane type-1 matrix metalloproteinase is a stromal activator of pro-gelatinase A. J. Cell Biol. 137, 67–77.